APPLIED MICROBIOLOGY, Sept 1967, p. 1045-1048
Vol. 15, No. 5

Microbiological Burden on the Surfaces of Explorer XXXIII Spacecraft[1]

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Received for publication 20 February 1967

The Explorer XXXIII Spacecraft (Anchored Interplanetary Monitoring Platform, or AIMP) was decontaminated to prevent gross contamination of the moon with terrestrial microorganisms. Assay of the total spacecraft surface before and after decontamination showed that the decontamination procedure reduced the viable microbiological burden from 1.40×10^6 to 3.60×10^4. However, assembly of parts which were not decontaminated for engineering reasons or were not assembled under clean-room conditions increased the viable microbial burden at the time of launch to 2.62×10^5.

Although the National Aeronautics and Space Administration (NASA) requires sterilization of planetary landing spacecraft, the sterilization requirement was rescinded for lunar landing spacecraft in 1963 and superseded by a new requirement that lunar spacecraft should be decontaminated to the fullest practical extent (NASA Management Manual 4-4-1, Chapter 4, p. 1-5). This change was prompted by the view that life as we know it could not exist on the moon. The lunar surface appears to be well below the freezing point of water except for the topmost few centimeters, which are exposed to high vacuum and radiation as well as alternately to subzero and very high temperatures (5). However, some workers (4, 8) believe there is a remote chance that terrestrial microorganism could grow on the moon. Lederberg and Cowie (6) also warned that gross contamination of the moon could interfere with attempts to determine whether organic substances found on the moon are native or were brought from the earth.

Sterilization of planetary landing spacecraft is done primarily for two reasons: (i) contamination of a planet with terrestrial microorganisms could frustrate efforts to demonstrate the existence of extraterrestrial life; (ii) overgrowth of a planet with terrestrial microorganisms might alter indigenous extraterrestrial life. Although a decontamination program cannot alone produce a sterile spacecraft, it can increase the level of confidence of a sterilization program by reducing the microbiological burden to a level that will ensure the effectiveness of a given sterilization cycle. Reduction of the contamination could also shorten the sterilization time and thus reduce the thermal stresses on spacecraft components.

Hobby (3) estimated that the probable spacecraft contamination level is 10^9 organisms. However, the microbiological burden of a typical, completely assembled spacecraft, before and after decontamination, has not been determined under actual working conditions.

The purpose of the decontamination procedure reported here was to reduce the microbiological decontamination on the Anchored Interplanetary Monitoring Platform (AIMP), or Explorer XXXIII, to the lowest possible level, to prevent gross contamination of the lunar surface if the spacecraft should impact on the moon.

The primary objective of the AIMP spacecraft is to measure interplanetary magnetic fields, solar plasma, energetic particles, and micrometeorite fluxes in the vicinity of the moon.

MATERIALS AND METHODS

Decontamination. All surfaces of the spacecraft were decontaminated with 90% isopropyl alcohol (Fisher Scientific Co., Pittsburgh, Pa.) mixed with 10% distilled water.

The spacecraft was first precleaned with the alcohol in the receiving area of the clean-room complex. It was then taken through an air shower and into a class 100 laminar crossflow clean room (1, Federal Standard No. 209, p. 8, Government Printing Office, Washington, D.C.) where assembly was accomplished in the following manner.

[1] This research was conducted for and in cooperation with the Spacecraft Integration and Sounding Rocket Division assigned the responsibility for decontamination of the spacecraft.

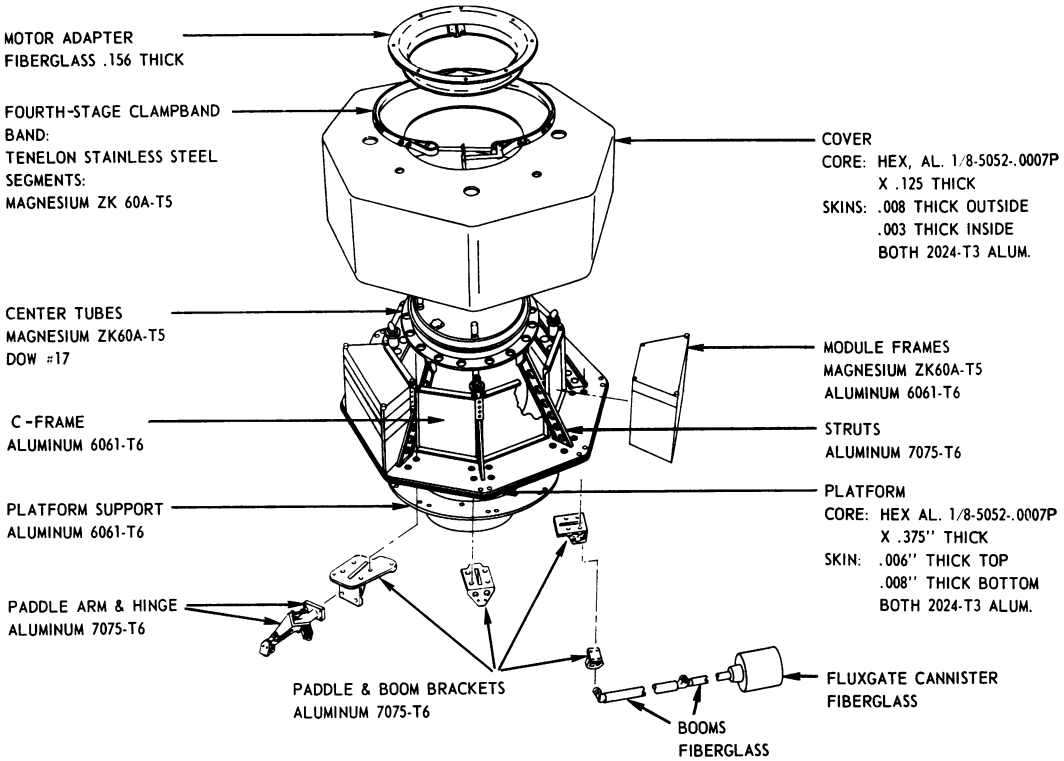

FIG. 1. *Explorer XXXIII spacecraft showing spacecraft configuration and structural materials.*

The spacecraft was placed approximately 1 ft (30 cm) in front of the bank of air inlet filters where it was decontaminated again prior to assembly of all parts; alcohol was applied with ester-type polyurethane foam wipers (cleanline wipers R) just before a surface was occluded. Standard clean-room operating procedure was observed at all times. All personnel remained downsteam of the spacecraft at all times during the assembly. Clean-room clothing (lint-free coveralls, cap, hood, face mask, booties, and sterile rubber gloves) was worn by all personnel. Passage of personnel through an air shower prior to entering the clean room was mandatory.

Sampling procedure. Several swab samples of each area were taken before and after decontamination; the latter samples were taken just before a surface was occluded. Sterile cotton swabs wetted with sterile distilled water were used. Quantitative counts were obtained by sampling 4 inch^2 (26 cm^2) areas outlined by sterile kraft paper templates which had been prepared to fit the configuration of the surface to be sampled.

The swabs were aseptically broken off into screw-cap tubes (125 $\times$ 20 mm) containing 10 ml of sterile distilled water. Each tube was vigorously shaken until the cotton was removed from the applicator stick and uniformly distributed in the solution. Viable-cell counts were made by culturing 4-ml samples in duplicate by routine bacteriological procedures.

Media. All plate counts were made on Difco Tryptic Soy Agar.

Incubation. All culture plates were incubated aerobically at 32 C for 72 hr. Negative plates were incubated for an additional 24 hr.

Clean rooms. The spacecraft was assembled in a class 100 laminar cross-flow room, with one exception: the last phase of the assembly was accomplished in a portable class 100 curtained laminar down-flow room (W. J. Whitfield et al., *unpublished data*) which was set up in a class 10,000 laminar cross-flow room.

RESULTS

The configuration of the Explorer XXXIII spacecraft is shown in Fig. 1 and 2.

Table 1 lists the microbiological contamination detected on surfaces of the spacecraft during the seven phases of the assembly. The seventh and last phase of the assembly took place at Cape Kennedy shortly before launch. The number of microorganisms before and after decontamination in assembly phase seven does not include the four solar paddles. Unlike the rest of the spacecraft, the solar paddles were cleaned with methyl ethyl ketone (MEK) because of engineering requirements, one of which involved the thermal properties of the solar cells. The MEK was not effective in reducing the microbial contamination, since one paddle had a much higher count after cleaning than before, and there was no significant

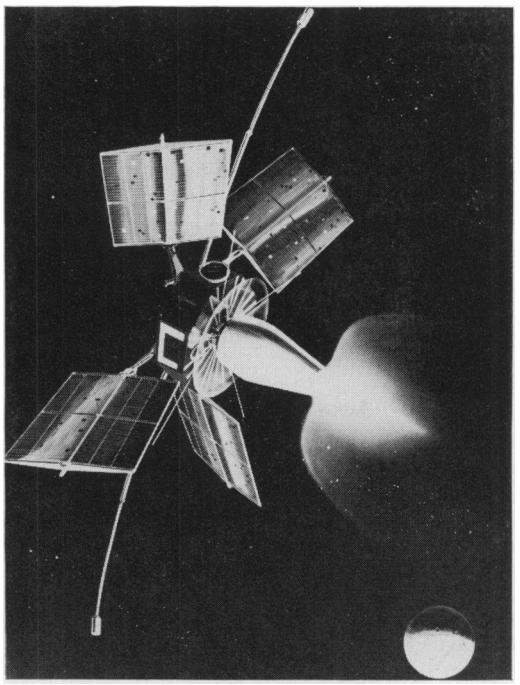

FIG. 2. *Artist's concept of the Explorer XXXIII spacecraft.*

reduction on the other three paddles. The total count on the paddles after cleaning is shown in Table 2.

Table 2 shows the microbial burden on the completely assembled spacecraft after decontamination. The multilayered (23 layers) thermal blankets were heat-sterilized at 135 C for 24 hr and sampled after installation on the spacecraft. Laboratory tests showed that the blankets were sterile, so it was concluded that the contamination detected occurred during their installation in the spin facility, a nonclean-room area. Since it was not possible to sample the entire blanket, it was necessary to extrapolate the count obtained from sampling a small area of the top layer to the entire surface area of the blanket. This probably resulted in a higher contamination level than actually occurred (Table 2). Because the solar paddles and thermal blankets were not decontaminated in the usual manner, the contamination on these surfaces was added to that detected on the spacecraft rather than included in the average, and the result was a "worse case" contamination level.

DISCUSSION

Although the "damp swab" procedure, like many other recovery procedures, is not capable of 100% recovery of microorganisms, it is quite

TABLE 1. *Microbial contamination on surfaces of Explorer XXXIII spacecraft during assembly*

Assembly pahse	Date	Surface area sampled (inch²)	Viable organisms per ft²	
			Before decontamination	After decontamination
1	11–30–65	135	21,174	563
2	12–13–65	134	22,388	230
3	12–23–65	365	1,000	75
4	1–3–66	154	2,125	90
5	2–7–66	416	1,299	219
6	5–2–66 to 5–18–66	431	416	33
7	6–9–66 to 6–29–66	208	854	94
Avg/ft²			7,036	186
Avg/spacecraft[a]			1,364,984	36,084

[a] Total area was 194 ft². Count excludes solar paddles and thermal blankets.

TABLE 2. *Determination of the total microbial burden on surfaces of the completely assembled explorer XXXIII spacecraft at time of launch*

Surface	Surface Area[a] (ft²)	Viable organisms
Spacecraft	194[b]	36,084
Solar paddles (four)	39	208,600
Thermal blankets (main and circular)	293	17,352
Total (sum)	526	262,036

[a] Surface areas determined by the Mechanical Systems Branch.

[b] Includes area of internal components.

reproducible, easy to use, and lends itself to any surface configuration.

Decontamination of the spacecraft surfaces assayed (194 ft²) reduced the microbial burden by approximately two orders of magnitude (Table 1). However, assembly of the four solar panels which were not decontaminated with isopropyl alcohol increased the burden by 2.08×10^5 organisms. Although the thermal blankets, which make up more than half of the total surface area, were sterilized, contamination of the outer-most layers during assembly contributed 1.7×10^4 organisms. This points out the need for components which can be sterilized and the necessity for aseptic, or clean-room assembly of spacecraft.

No attempt was made by us to determine the internal component burden because it was im-

practical. However, an estimate of the internal component burden could be derived by extrapolating the numbers reported by other workers (2) to similar components in the Explorer XXXIII spacecraft. Such an estimate for 17,885 components (resistors, capacitors, transformers, and other electronic devices) and 14,727 ml of foam in the Explorer XXXIII spacecraft would range from 1.15×10^6 to 1.35×10^7 microorganisms.

The single greatest contribution to this extremely high estimate of the internal component burden was from transformers. It was estimated that the internal burden of transformers ranged from 10,000 to 100,000 microorganisms (2). This would mean that, for 97 torroidal transformers in the Explorer XXXIII spacecraft, the microbial burden from this source alone would range from 9.7×10^5 to 9.7×10^6.

It is possible that the above estimates are several orders of magnitude too high. A recent preliminary investigation of torroidal transformers indicated little or no internal microbial contamination (7). The only viable microorganisms recovered were from external surfaces, and the count was less than 100 organisms. The implication of these results is important, because in contrast to the previous reference it indicates that the internal component burden may be a small contribution to the total contamination of a spacecraft.

If this is the case, the numbers per square foot of surface area measured on the Explorer XXXIII spacecraft (Table 1) may be an accurate index of the microbial contamination of similar spacecraft. By using these numbers, for example, the estimated microbial burden of the completely assembled Explorer XXXIII spacecraft (526 ft^2) would be 3.7×10^6 before decontamination and 9.78×10^4 after decontamination. It is possible that die-off during orbit in space for 6 months or more may reduce the contamination further by as much as two orders of magnitude (8). This would lower the contamination to an estimated 10^3 microorganisms at lunar impact.

Estimations such as these have significant implications for the sterilization of planetary spacecraft because the duration of sterilization cycles is based on an estimate of the number of microorganisms present at the start of the cycle. It is clear, however, that a great deal of data must be compiled before such estimates can be made with some degree of accuracy.

While no attempt was made to identify the microorganisms detected, the following types were noted: *Staphylococcus* sp., *Bacillus* sp., *B. globigii*, *Sarcina* sp., *Flavobacterium* sp., *Aspergillus* sp., and *Penicillium* sp. All are airborne microorganisms commonly found on the skin of humans.

Decontamination of the Explorer XXXIII was significant for several reasons: (i) this was the first spacecraft which was completely monitored and decontaminated through all phases of assembly; (ii) an applied working estimate of the microbial burden of a spacecraft was obtained; (iii) several biological and engineering problems were identified; (iv) the importance of the microbiologist-engineer team was recognized, as well as the necessity for close collaboration between the two disciplines and of a mutual understanding of various problems.

ACKNOWLEDGMENTS

The author thanks P. G. Marcotte and F. N. LeDoux, both of Goddard Space Flight Center Spacecraft Integration and Sounding Rocket Division, for the opportunity to work on the spacecraft and for their cooperation in the monitoring effort, and A. J. Pierro and his associates of the Goddard Space Flight Center Spacecraft Integration and Sounding Rocket Division for their conscientious efforts to decontaminate the spacecraft.

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